FEBS Letters 546 (2003) 374–378 FEBS 27398

The SUMO pathway is required for selective degradation of DNA topoisomerase IIβ induced by a catalytic inhibitor ICRF-193[1]

Sevim Isik[a,2], Kuniaki Sano[b,2], Kimiko Tsutsui[b], Masayuki Seki[c], Takemi Enomoto[c], Hisato Saitoh[d], Ken Tsutsui[a,*]

[a] *Department of Molecular Biology, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8558, Japan*
[b] *Department of Neuroanatomy and Neurobiology, Okayama University Graduate School of Medicine and Dentistry, Okayama 700-8558, Japan*
[c] *Molecular Cell Biology Laboratory, Graduate School of Pharmaceutical Sciences, Tohoku University, Sendai 980-8578, Japan*
[d] *Department of Regeneration Medicine, Institute of Molecular Embryology and Genetics, Kumamoto University, Kumamoto 860-0811, Japan*

Received 12 March 2003; revised 26 May 2003; accepted 28 May 2003

First published online 11 June 2003

Edited by Ulrike Kutay

Abstract DNA topoisomerase I and II have been shown to be modified with a ubiquitin-like protein SUMO in response to their specific inhibitors called 'poisons'. These drugs also damage DNA by stabilizing the enzyme–DNA cleavable complex and induce a degradation of the enzymes through the 26S proteasome system. A plausible link between sumoylation and degradation has not yet been elucidated. We demonstrate here that topoisomerase IIβ, but not its isoform IIα, is selectively degraded through proteasome by exposure to the catalytic inhibitor ICRF-193 which does not damage DNA. The β isoform immunoprecipitated from ICRF-treated cells was modified by multiple modifiers, SUMO-2/3, SUMO-1, and polyubiquitin. When the SUMO conjugating enzyme Ubc9 was conditionally knocked out, the ICRF-induced degradation of topoisomerase IIβ did not occur, suggesting that the SUMO modification pathway is essential for the degradation.

Key words: Ubiquitin-like protein; SUMO; DNA topoisomerase II; Proteasome

1. Introduction

Recent studies on post-transcriptional protein modification by proteinaceous modifiers, ubiquitin and ubiquitin-like proteins (UBLs) have opened an avenue to novel regulatory pathways in living cells [1,2]. SUMO (small ubiquitin-like modifier) is a highly conserved protein in eukaryotes and the most-studied UBL to date. Although it shares only moderate sequence identity with ubiquitin, their conjugation pathways have extensive similarities. Yeast and invertebrates have a single SUMO (also called SMT3) but three isoforms, termed SUMO-1, SUMO-2, and SUMO-3, have been identified in vertebrates. The latter two are highly homologous to each other (94% identity) but diverge significantly from SUMO-1 (~50% identity). As compared to SUMO-1, much less information is available at present on the conjugation partners of SUMO-2/3. While selective degradation of multi-ubiquitinated proteins by the 26S proteasome has now been established, downstream events of sumoylation are less obvious. In some cases, SUMO modification occurs at the same lysine residues that are used for ubiquitination, thus inhibiting multi-ubiquitination and preventing the protein from degradation by proteasome [3]. Conversely, modification by SUMO has not yet been reported to accelerate protein degradation.

Camptothecin and VM-26, often referred to as topoisomerase poisons, are representative inhibitors of type I (topo I) and type II (topo II) topoisomerases, respectively. These drugs bind specifically to each enzyme and stabilize the topo–DNA covalent intermediates (cleavable complex) by preventing the religation of transiently cleaved DNA strands [4,5]. ICRF-193 and other bisdioxopiperazines, classified as 'catalytic' inhibitors of topo II, do not interfere with the DNA rejoining step but bind to the closed-clamp form of the enzyme–ATP complex to trap the DNA strands topologically between the dimeric subunits [6]. It has been shown that topoisomerases undergo proteasome-mediated degradation in cells treated with topo poisons [7,8]. Since poisoned cells eventually generate enzyme-linked DNA double strand breaks that are toxic to cells [9], the induced reduction of enzyme level was thought to be a cellular defense mechanism [10].

Two isoforms of topo II, α and β, are present in vertebrates that are encoded by separate genes [11], whereas only one form is present in lower eukaryotes including the budding yeast *Saccharomyces cerevisiae*, in which sumoylation of topo II is involved in the control of centromeric chromatid cohesion [12]. Although topoisomerase inhibitors have been reported to induce sumoylation of both topo I and topo II [13,14], its biological significance remains largely unknown. These observations suggest a connection between sumoylation and degradation but this conjecture has not been fully tested. In the present communication, we demonstrate that topo IIβ is selectively degraded via proteasome by exposure to ICRF-193, a catalytic inhibitor which does not damage DNA, and that protein modification by SUMO can be an early signal for the degradation.

*Corresponding author. Fax: (81)-86-235 7103.
E-mail address: tsukken@cc.okayama-u.ac.jp (K. Tsutsui).

[1] The DT40 cell line used here (*Ubc9*$^{-/-/-}$tet*Ubc9*) will be available from RIKEN Cell Bank (http://www.rtc.riken.go.jp/CELL/HTML/RIKEN_Cell_Bank.html) by specifying the RCB number (RCB 1845) and the cell line name (delta Ubc9+cUbc9-DT40).
[2] These authors contributed equally to this work.

Abbreviations: SUMO, small ubiquitin-like modifier; topo I/II, DNA topoisomerase I/II; PAGE, polyacrylamide gel electrophoresis

0014-5793/03/$22.00
doi:10.1016/S0014-5793(03)00637-9

2. Materials and methods

2.1. Chemicals and antibodies

The following chemicals were obtained from the suppliers indicated in parentheses: ICRF-193 (Funakoshi), VP-16 (gift from Nippon Kayaku), MG132 (Calbiochem), camptothecin, leptomycin B and doxycycline hydrochloride (Sigma). Most compounds were dissolved in dimethyl sulfoxide, and stored at −20°C in small aliquots. S7 nuclease, protease inhibitor cocktail, and protein G-Sepharose were purchased from TaKaRa, Sigma, and Amersham Pharmacia Biotech, respectively.

Mouse monoclonal antibodies against human topo IIα (4E12) and topo IIβ (3B6) were prepared as described previously [15], and used for both Western blotting and immunoprecipitation experiments. 3B6 was cross-reactive with rat and chicken antigens. Rabbit polyclonal antibody to SUMO-1 (FL-101) was purchased from Santa Cruz Biotech. The anti-SUMO-2/3 polyclonal antibody was prepared as described [16]. Mouse monoclonal antibodies against polyubiquitin (FK2) and Ubc9 (N-15) were obtained from Affinity Research Products and Santa Cruz Biotech, respectively. IgG prepared from the serum of a progressive systemic sclerosis patient was used for anti-DNA topoisomerase I antibody. Anti-matrin 3 antibody was prepared from a rabbit immunized with a synthetic peptide.

2.2. Cell culture

HeLa cells were cultured in a humidified atmosphere of 5% CO_2 at 37°C in DMEM medium supplemented with 5% fetal calf serum (FCS) and 100 μg/ml kanamycin. Cerebellar granule cells were prepared from the cerebellum of 8-day-old Wistar rats and processed for primary culture as described previously [15]. Wild type DT40 cells and *Ubc9*$^{-/-/-}$tet*Ubc9* cells were cultured at 39.5°C under 5% CO_2 in RPMI 1640 supplemented with 10% heat-inactivated FCS plus 1% chicken serum and 60 μg/ml kanamycin [17].

2.3. Western blotting

Subconfluent HeLa cells or cerebellar granule cells at culture day 3 were treated as described in the figure legends. After treatments, cells were lysed in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) sample buffer (50 mM Tris–HCl: pH 6.8, 2 mM EDTA, 1% SDS, 1% 2-mercaptoethanol, 8% glycerol, and 0.025% bromophenol blue) added directly into culture dishes. Samples were sonicated briefly and boiled for 2 min before being subjected to electrophoresis. In the experiments where VP-16 or camptothecin was used, lysates were prepared by the alkali lysis procedure followed by digestion with S7 nuclease as described [7]. Samples were run in either 6% homogeneous or 2–15% gradient polyacrylamide gels, transferred onto polyvinylidene difluoride or nitrocellulose membranes by electroblotting, and processed for immunodetection with appropriate first and second antibodies according to a standard procedure. Detection of membrane-bound antibodies was done using an ECL kit (Amersham Pharmacia Biotech). Developed films were scanned for densitometric quantification of protein bands. When successive detection with different antibodies was attempted, the bound antibodies were stripped off the membrane each time by shaking the membrane in the antibody stripping buffer (2% SDS, 100 mM 2-mercaptoethanol, 62.5 mM Tris–HCl, pH 6.8) at 60°C for 30 min.

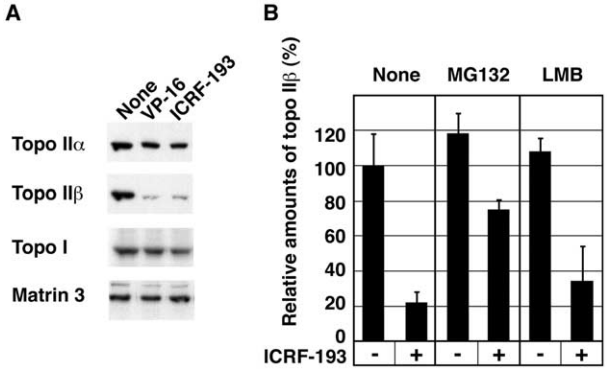

Fig. 1. Selective degradation of topo IIβ in HeLa cells treated with ICRF-193 and effects of inhibitors. A: Selective down-regulation of topo IIβ by topo II-specific inhibitors with different action mechanisms. Cell lysates prepared by alkaline solution from HeLa cells incubated for 2 h with topo II-specific inhibitors, 100 μM VP-16 or 10 μM ICRF-193, were treated with S7 nuclease and then subjected to Western blotting using antibodies against the proteins indicated. B: Effects of inhibitors on the ICRF-induced down-regulation of topo IIβ. HeLa cells were treated with the drugs indicated at the top for 1 h, in the presence (+) or absence (−) of 10 μM ICRF-193. These drugs were added 30 min before the addition of ICRF-193. Concentrations used: 20 μM, MG132; 10 ng/ml, LMB. Cell lysates were subjected to Western blotting to quantify topo IIβ. Densitometric values from film scanning were normalized to those of matrin 3, as an internal standard, whose expression level remained constant. Measurements were done on triplicate samples from separate dishes and were averaged. Amounts relative to the untreated control are shown with error bars.

2.4. Immunoprecipitation

Drug-treated cells were lysed in RIPA buffer (50 mM Tris–HCl, pH 7.5, 150 mM NaCl, 1% Nonidet P-40, 0.5% sodium deoxycholate, and 0.1% SDS) containing 2% protease inhibitor cocktail, at 4×10^6 cells/ml. Cell lysates were vortexed, sonicated briefly, left on ice for 30 min, and then spun at 14 000 rpm for 10 min at 4°C. After pre-clearing the supernatants (1 ml) with 50 μl of 50% protein G-Sepharose slurry, the same volume of protein G-Sepharose that had been pre-coated with 10 μg of anti-topo IIβ monoclonal IgG was added, and incubated with gentle rotary mixing at 4°C for 1 h. The suspension was centrifuged at 6000 rpm for 1 min, and the sedimented beads were washed three times with RIPA buffer. Immunoprecipitates were resuspended in SDS–PAGE sample buffer, boiled for 2 min, and subjected to electrophoresis.

3. Results

3.1. ICRF-193 induces proteasome-mediated degradation of DNA topoisomerase IIβ in different cell types

We have shown previously that topo IIβ, highly expressed in cerebellar granule neurons differentiating in vitro, is involved in the transcriptional induction of a subset of genes that are essential for mature neuronal functions [15]. In that study, we used the catalytic inhibitor ICRF-193 to suppress the activity of topo IIβ and found unexpectedly that the expression of topo IIβ was markedly reduced in the treated cells. Subsequent experiments have indicated that the reduction of topo IIβ is not accounted for by suppression of transcription or translation but is due to a specific degradation via the proteasome (data not shown).

Since granule cells cultured in vitro do not express topo IIα [15], we used HeLa cells, in which both topo II isoforms are expressed, to examine whether topo IIα is also affected by ICRF-193. A significant reduction in the topo IIβ amount was evident after the treatment with both topo II inhibitors, VP-16 (a topo poison) and ICRF-193, whereas topo IIα was not affected at all under these conditions (Fig. 1A). Levels of topo I or a protein associated with nuclear matrix (matrin 3) did not change by the treatment. As in granule cells, the ICRF-induced reduction in topo IIβ represented its degradation by proteasome which is susceptible to MG132 (Fig. 1B). Thus, the effect of ICRF-193 on topo IIβ degradation is very similar to that of VM-26 [8]. Because components of the proteasome system are present in both cytoplasm and nucleus [18], cells were treated with a nuclear protein export inhibitor, leptomycin B (LMB), to see whether topo IIβ degradation occurs after export to the cytoplasm. LMB had little effect on the degradation, indicating that topo IIβ is degraded by nuclear proteasome (Fig. 1B).

Granule cells in primary culture are terminally differentiating without further proliferation whereas HeLa cells are proliferating cells. Although the ICRF-induced topo IIβ degradation occurs in both cell types, the rate of degradation appears to differ significantly. A time course analysis revealed that topo IIβ degrades much faster in HeLa cells compared to granule cells (Fig. 2). The degradation appeared to proceed in two phases, rapid and slow. The half-degradation time ($t_{1/2}$) for the rapid phase was 10 min and 53 min in HeLa cells and granule cells, respectively.

3.2. *Multiple modification of DNA topo IIβ in granule cells treated with ICRF-193*

We used granule cells to analyze the modification status of topo IIβ for the following reasons. Unlike in HeLa cells, the modified forms of topo IIβ are readily identifiable in granule cells. The slower time course of topo IIβ degradation in granule cells is also advantageous to discriminate the chain of events. Topo IIβ immunoprecipitates prepared at various times of incubation with ICRF-193 were separated in a gradient gel to attain a better resolution in the high-molecular-weight region. After blotting, the same membrane was probed with antibodies to topo IIβ, SUMO-2/3, SUMO-1, and poly-ubiquitin. These antibodies revealed a ladder of discrete bands overlying on smears (Fig. 3). The bands right above the topo IIβ band that are detected with anti-SUMO-2/3 antibody appeared first as early as after 5 min of ICRF treatment and decreased after 15 min. Bands reactive to anti-SUMO-1 increased abruptly at 15 min and continued to increase afterwards. The smearing polyubiquitinated bands followed a similar time course although they reached a higher molecular weight region. It should be noted that these smears are hardly discernible in the top panel (topo IIβ) because of the much lower epitope density. Since the anti-ubiquitin antibody used here reacts specifically with polymerized ubiquitin, the modified topo IIβ detected by the antibody is likely to be targeted directly to proteasome.

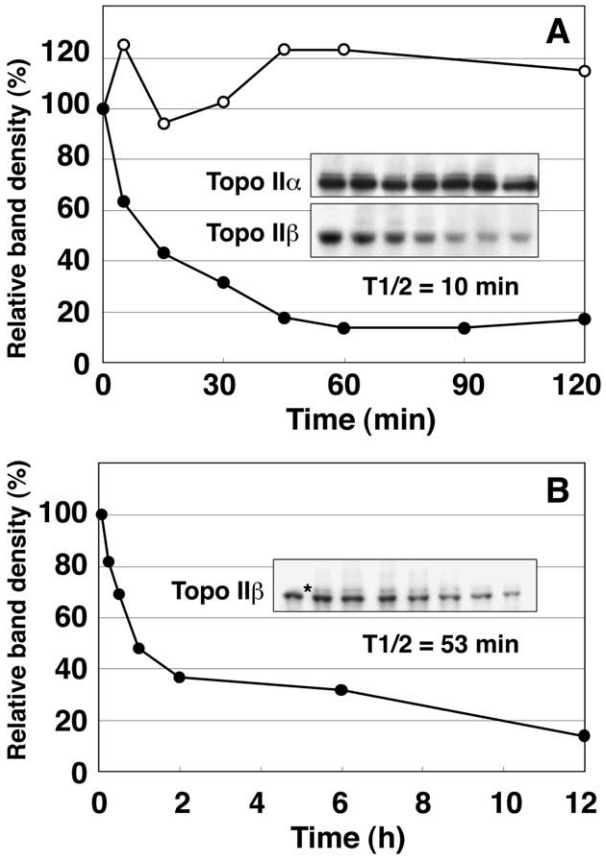

Fig. 2. Differential time course of topo IIβ degradation in HeLa cells and granule cells. Cultured cells treated with ICRF-193 were lysed at increasing time points and subjected to Western blotting to detect topo II isoforms (shown in insets). Band densities were quantified by densitometry and the data relative to zero time were plotted against incubation time. A: HeLa cells treated with 10 μM ICRF-193. Open circles, topo IIα; closed circles, topo IIβ. Time points shown in the inset are 0, 5, 15, 30, 45, 60, and 90 min. $t_{1/2}$ for the rapid phase of topo IIβ degradation was estimated from a semi-logarithmic plot. B: Granule cells treated with 30 μM ICRF-193. Closed circles designate topo IIβ. Time points shown in the inset are 0, 5, 15, 30, 60, 120, 360, and 720 min. Note that the upper band (marked by the asterisk) already appears after 5 min of treatment.

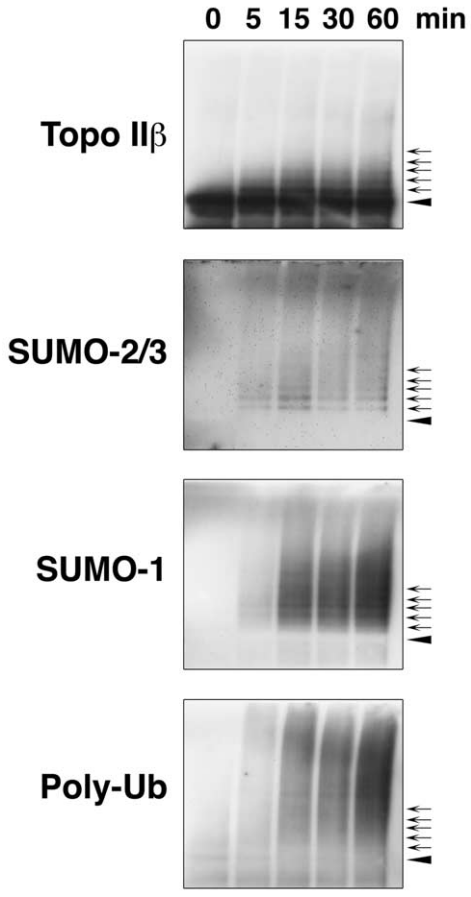

Fig. 3. Distinct modification patterns of topo IIβ in granule cells treated with ICRF-193. Granule cells treated with 30 μM ICRF-193 were lysed with RIPA buffer at increasing time points and subjected to immunoprecipitation with anti-topo IIβ antibody. Immunoprecipitates were run in SDS–PAGE gradient gel, and then subjected to Western blotting to detect the proteins indicated on the left with corresponding antibodies. The same membrane was used throughout by stripping off antibodies between detections (see Section 2). Modified protein bands in the high-molecular-weight region are labeled on the right (arrows). The unmodified topo IIβ band is indicated by arrowheads. Incubation times are shown at the top.

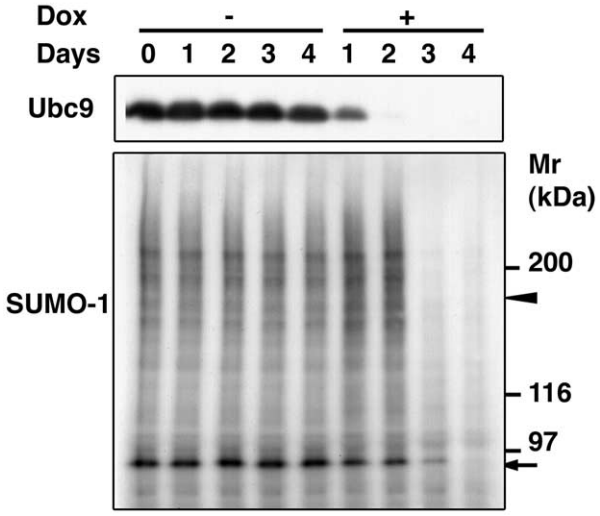

Fig. 4. Down-regulation of Ubc9 and sumoylation in a Ubc9-deficient chicken DT40 cell line containing an exogenous *Ubc9* gene that can be turned off by doxycycline. The mutant cells ($Ubc9^{-/-/-}$tet*Ubc9*) were incubated in the presence (+) or absence (−) of 10 ng/ml doxycycline (Dox) for the days indicated, and the expression of Ubc9 or proteins modified with SUMO-1 was analyzed by Western blotting using specific antibodies. Positions of topo IIβ (arrowhead), RanGAP1 (arrow), and molecular size markers are indicated.

3.3. *The SUMO conjugating enzyme Ubc9 is essential for the degradation of DNA topo IIβ*

Ubc9 is the only enzyme identified so far to conjugate SUMO isoforms to target proteins [19]. To test whether sumoylation is an essential modification for ICRF-induced topo IIβ degradation, we used a mutant chicken DT40 cell line which lacks the endogenous *Ubc9* gene but contains a *Ubc9* transgene under the control of a tetracycline-repressible promoter ($Ubc9^{-/-/-}$tet*Ubc9* cells). When the *Ubc9* mutant cells were treated with doxycycline for 2 days, Ubc9 expression was reduced markedly as expected, and abolished completely after 3 days (Fig. 4). The sumoylated proteins detected by anti-SUMO-1 antibody started to decrease later and became almost undetectable on day 4 of doxycycline treatment, including the 90 kDa protein which is most likely RanGAP1 modified by SUMO-1 [20]. These results are consistent with the original character of $Ubc9^{-/-/-}$tet*Ubc9* cells reported previously [17].

Wild type DT40 cells and $Ubc9^{-/-/-}$tet*Ubc9* cells cultured for 4 days in the presence or absence of doxycycline were next treated with ICRF-193 and levels of topo IIβ or sumoylated proteins were determined by immunoblotting (Fig. 5A). In wild type DT40 cells, as in HeLa cells and granule cells, ICRF-193 induced degradation of topo IIβ with a concomitant increase in the high-molecular-weight proteins recognized by antibody to SUMO-1. The topo IIβ degradation was also susceptible to MG132 (not shown). In doxycycline-treated $Ubc9^{-/-/-}$tet*Ubc9* cells, however, ICRF-193 induced neither topo IIβ degradation nor an increase in sumoylated proteins. The result was confirmed by densitometric quantification using triplicate samples (Fig. 5B). To see whether the ubiquitin–proteasome pathway is intact in the absence of Ubc9 expression, the camptothecin-induced degradation of topo I was examined (Fig. 5C). The induced reduction of topo I, which was suppressed by MG132, occurred to the same extent even

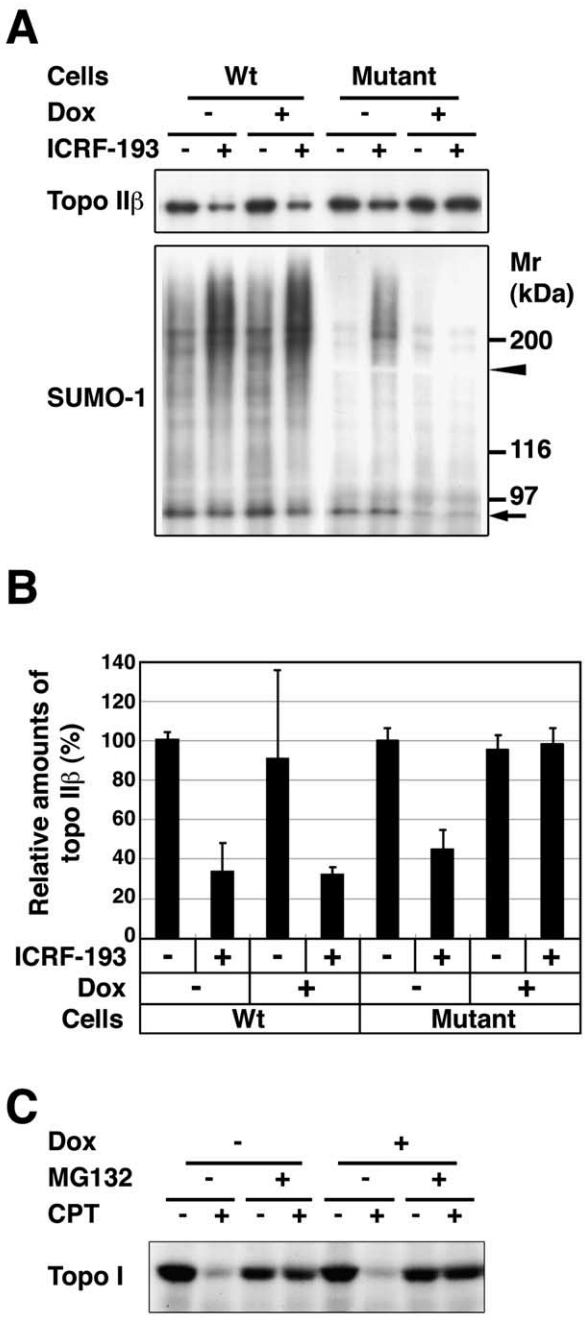

Fig. 5. Ubc9 is essential for the degradation of topo IIβ induced by ICRF-193. A: After incubating the wild type DT40 cells (wt) or $Ubc9^{-/-/-}$tet*Ubc9* cells (mutant) in the presence (+) or absence (−) of 10 ng/ml doxycycline for 4 days, cells were incubated with (+) or without (−) 10 μM ICRF-193 for 2 h. Levels of topo IIβ and sumoylated proteins were analyzed by Western blotting using specific antibodies. B: Three independent reactions were done under the same experimental conditions shown above. After normalization to an internal standard, densitometric readings for topo IIβ from triplicate samples were averaged and the amounts relative to untreated controls are shown with error bars. C: $Ubc9^{-/-/-}$tet*Ubc9* cells treated as above were incubated for 4 h in the presence (+) or absence (−) of 25 μM camptothecin (CPT). MG132 (30 μM) was added 30 min prior to the start of incubation. Cell lysates were prepared by the alkali lysis procedure followed by S7 nuclease digestion and then topo I was detected by Western blotting.

in the presence of doxycycline, indicating that the proteolytic system itself is still unimpaired under these conditions. Therefore, it is now clear that sumoylation is a prerequisite for ICRF-induced topo IIβ degradation.

4. Discussion

We have shown in the present study that topo IIβ degradation can be induced by the catalytic inhibitor ICRF-193 which does not damage DNA. Since the topo II protein clamp on DNA induced by the drug could be an obstacle against various DNA transactions, the same consequence, namely the accelerated degradation that is caused by topo II poisons, may be expected also for catalytic inhibitors. A similar observation was made by others recently [21].

The ICRF-induced multiple modification of topo IIβ appeared to occur in a cascade-like manner (Fig. 3). SUMO-2/3 conjugation may be particularly important because it preceded that of SUMO-1 and polyubiquitin. Consistent with this view is a rapid and extensive conjugation of SUMO-2/3 to high-molecular-weight proteins following various protein-damaging stresses such as high temperature, oxidative agent, and ethanol [16]. It is an interesting possibility that SUMO-2/3 conjugation to denatured proteins somehow triggers subsequent events toward their disposal through the proteasome system. Unlike SUMO-1, SUMO-2/3 has a consensus motif for autosumoylation and can form polymeric chains on protein substrates [22]. Formation of heteromultimers of different SUMO isomers is also feasible, where SUMO-1 may serve as a chain terminator. Whether topo IIβ is modified by polymeric chains of SUMO or whether the same molecule of topo IIβ is simultaneously modified with SUMO and ubiquitin remains to be shown. The SUMO moiety might be removed by deconjugating isopeptidase before ubiquitination as suggested by the transient nature of SUMO-2/3 conjugates (Fig. 3).

Analysis of Ubc9-deficient DT40 cells demonstrated unambiguously that SUMO modification is essential for ICRF-induced degradation of topo IIβ (Fig. 5). Although our data do not exclude the possible involvement of other sumoylation targets, the selective degradation of topo IIβ may imply that potential sumoylation sites are different between topo II isoforms. The topo IIβ–inhibitor–DNA ternary complex may be a preferential target of Ubc9. Whether SUMO conjugation facilitates the dissociation of topo IIβ clamps from DNA is still unclear. An interesting discovery in this regard is that the affinity of thymine-DNA glycosylase to abasic site DNA is significantly reduced when the enzyme is modified by both SUMO-1 and SUMO-2/3, resulting in an increased enzymatic turnover [23]. In contrast to topo IIβ, it appears that SUMO modification plays little role in camptothecin-induced topo I degradation which occurs independent of Ubc9 (Fig. 5C). This is consistent with the previous report showing that the rate of degradation was not altered even when all the major sumoylation sites of topo I were disrupted by mutation [24].

As for the relationship between sumoylation and ubiquitination, our results imply that these modifications are cooperating, instead of antagonizing [3], to direct the stalled topo IIβ to destruction via proteasome, suggesting a possible cross-talk between these pathways in the repair of damaged chromatin.

Acknowledgements: We thank Dr. A. Kikuchi (Nagoya University) for anti-topo II monoclonal antibodies. Polyclonal antibody to matrin 3 was kindly provided by Dr. R. Kuwano (Niigata University). We are also grateful to Ms. R. Takeuchi-Iwata for technical assistance. This work was supported by Grants-in-Aid for Scientific Research from the Ministry of Education, Science, Sports and Culture of Japan (Project 11239206).

References

[1] Yeh, E.T., Gong, L. and Kamitani, T. (2000) Gene 248, 1–14.
[2] Müller, S., Hoege, C., Pyrowolakis, G. and Jentsch, S. (2001) Nat. Rev. Mol. Cell Biol. 2, 202–210.
[3] Desterro, J.M., Rodriguez, M.S. and Hay, R.T. (1998) Mol. Cell 2, 233–239.
[4] Hsiang, Y.H., Hertzberg, R., Hecht, S. and Liu, L.F. (1985) J. Biol. Chem. 260, 14873–14878.
[5] Chen, G.L., Yang, L., Rowe, T.C., Halligan, B.D., Tewey, K.M. and Liu, L.F. (1984) J. Biol. Chem. 259, 13560–13566.
[6] Roca, J., Ishida, R., Berger, J.M., Andoh, T. and Wang, J.C. (1994) Proc. Natl. Acad. Sci. USA 91, 1781–1785.
[7] Desai, S.D., Liu, L.F., Vazquez-Abad, D. and D'Arpa, P. (1997) J. Biol. Chem. 272, 24159–24164.
[8] Mao, Y., Desai, S.D., Ting, C.Y., Hwang, J. and Liu, L.F. (2001) J. Biol. Chem. 276, 40652–40658.
[9] Wu, J. and Liu, L.F. (1997) Nucleic Acids Res. 25, 4181–4186.
[10] Desai, S.D., Li, T.K., Rodriguez-Bauman, A., Rubin, E.H. and Liu, L.F. (2001) Cancer Res. 61, 5926–5932.
[11] Austin, C.A. and Marsh, K.L. (1998) BioEssays 20, 215–226.
[12] Bachant, J., Alcasabas, A., Blat, Y., Kleckner, N. and Elledge, S.J. (2002) Mol. Cell 9, 1169–1182.
[13] Mao, Y., Sun, M., Desai, S.D. and Liu, L.F. (2000) Proc. Natl. Acad. Sci. USA 97, 4046–4051.
[14] Mao, Y., Desai, S.D. and Liu, L.F. (2000) J. Biol. Chem. 275, 26066–26073.
[15] Tsutsui, K., Tsutsui, K., Sano, K., Kikuchi, A. and Tokunaga, A. (2001) J. Biol. Chem. 276, 5769–5778.
[16] Saitoh, H. and Hinchey, J. (2000) J. Biol. Chem. 275, 6252–6258.
[17] Hayashi, T., Seki, M., Maeda, D., Wang, W., Kawabe, Y., Seki, T., Saitoh, H., Fukagawa, T., Yagi, H. and Enomoto, T. (2002) Exp. Cell Res. 280, 212–221.
[18] Floyd, Z.E., Trausch-Azar, J.S., Reinstein, E., Ciechanover, A. and Schwartz, A.L. (2001) J. Biol. Chem. 276, 22468–22475.
[19] Hay, R.T. (2001) Trends Biochem. Sci. 26, 332–333.
[20] Matunis, M.J., Coutavas, E. and Blobel, G. (1996) J. Cell Biol. 135, 1457–1470.
[21] Xiao, H., Mao, Y., Desai, S.D., Zhou, N., Ting, C.Y., Hwang, J. and Liu, L.F. (2003) Proc. Natl. Acad. Sci. USA 100, 3239–3244.
[22] Tatham, M.H., Jaffray, E., Vaughan, O.A., Desterro, J.M., Botting, C.H., Naismith, J.H. and Hay, R.T. (2001) J. Biol. Chem. 276, 35368–35374.
[23] Hardeland, U., Steinacher, R., Jiricny, J. and Schar, P. (2002) EMBO J. 21, 1456–1464.
[24] Rallabhandi, P., Hashimoto, K., Mo, Y.Y., Beck, W.T., Moitra, P.K. and D'Arpa, P. (2002) J. Biol. Chem. 277, 40020–40026.